

WE CLAIM:

1. An artificially produced peptide which neutralizes a biological activity of interleukin-18, comprising a part or the whole of the amino acid sequences of variable regions in anti-interleukin-18 antibody.
2. The peptide of claim 1, wherein the anti-interleukin-18 antibody is a monoclonal antibody.
3. The peptide of claim 1, wherein the anti-interleukin-18 antibody is against human or mouse interleukin-18 as antigen.
4. The peptide of claim 1, which suppresses inflammation induced by the biological activity of interleukin-18.
5. The peptide of claim 1, wherein the variable regions comprise the amino acid sequences of SEQ ID NOs:1 and 2.
6. The peptide of claim 1, which comprises a part or the whole of the amino acid sequences of complementarity determining regions in the variable regions.
7. The peptide of claim 1, which comprises a part or the whole of the amino acid sequences of SEQ ID NOs:3 to 8.
8. The peptide of claim 1, which has an amino acid sequence selected from the group consisting of SEQ ID NOs:9 and 10.
9. The peptide of claim 1, which is in the form of a humanized antibody.
10. A DNA which codes for the peptide of claim 1.
11. The DNA of claim 10, which comprises a part or the whole of a nucleotide sequence selected from the group consisting

Rule 1.12b

DS

4/9/03

<sup>12</sup>

~~10.~~ The peptide of claim 1, wherein the interleukin-18-neutralizing activity of said peptide per antigen-binding site is substantially equivalent to that of the parental antibody.

<sup>13</sup>

~~11.~~ A composition comprising as an effective ingredient the peptide of claim <sup>12</sup>~~10~~ and a pharmaceutically acceptable carrier.

<sup>14</sup>

~~12.~~ A method to treat a living body for preventing, alleviating, or remedying a disease selected from the group consisting of asthma, graft-versus-host disease, rheumatoid arthritis, and sepsis, said method comprising administering an effective amount of the composition of claim <sup>13</sup>~~11~~ to the living body.

<sup>15</sup>

~~13.~~ A method to treat a living body in need of autoimmunity, immunosuppressive, or anti-inflammatory treatment, said method comprising administering an effective amount of the composition of claim <sup>13</sup>~~11~~ to the living body.

<sup>16</sup>

~~14.~~ The peptide of claim 1, wherein the interleukin-18-neutralizing activity of said peptide per antigen-binding site is not lower than that of an immunoglobulin molecule comprising the amino acid sequences of SEQ ID NOs:1 and 2 as the light and heavy chain variable regions respectively.

<sup>17</sup>

~~15.~~ A composition comprising as an effective ingredient the peptide of claim <sup>16</sup>~~14~~ and a pharmaceutically acceptable carrier.

<sup>18</sup>~~16~~. A method to treat a living body for preventing, alleviating, or remedying a disease selected from the group consisting of asthma, graft-versus-host disease, rheumatoid arthritis, and sepsis, said method comprising administering an effective amount of the composition of claim <sup>17</sup>~~15~~ to the living body.

<sup>19</sup>~~17~~. A method to treat a living body in need of autoimmunity, immunosuppressive, or anti-inflammatory treatment, said method comprising administering an effective amount of the composition of claim <sup>17</sup>~~15~~ to the living body.

<sup>20</sup>~~18~~. The peptide of claim 1, which comprises, as parts of the variable regions in an anti-interleukin-18 antibody, the complementarity determining regions in the light and heavy chain variable regions, wherein not more than 30% of the amino acids of each complementarity determining region are optionally replaced by different amino acids.

<sup>21</sup>~~19~~. The peptide of claim 1, wherein said variable regions are of the same antibody molecule.

<sup>22</sup>~~20~~. The peptide of claim 1, wherein each of the amino acid sequences comprising a part or the whole of the variable regions in an anti-interleukin-18 antibody exhibits the interleukin-18-neutralizing activity when linked with the amino acid sequence of SEQ ID NO:1 or 2 via a suitable linker in a single chain polypeptide.

<sup>23</sup>~~21~~. The peptide of claim 1, which comprises as a light chain variable region the amino acid sequence of SEQ ID

NO:1 or a fragment thereof and as a heavy chain variable region the amino acid sequence of SEQ ID NO.2 or a fragment thereof, wherein each of said fragments exhibits the interleukin-18-neutralizing activity when linked with the amino acid sequence of SEQ ID NO:1 or 2 via a suitable linker in a single chain polypeptide.

<sup>24</sup> 22. A DNA which codes for the peptide of claim 1.

<sup>25</sup> 23. The DNA of claim <sup>24</sup> 22, which comprises a part or the whole of a nucleotide sequence selected from the group consisting of SEQ ID NOS:11 and 12 and their complementary sequences.

<sup>26</sup> 24. The DNA of claim <sup>24</sup> 22, which comprises a part or the whole of a nucleotide sequence selected from the group consisting of SEQ ID NOS:13 to 18 and their complementary sequences.

<sup>27</sup> 25. The DNA of claim <sup>24</sup> 22, which has a nucleotide sequence selected from the group consisting of SEQ ID NOS:19 and 20 and their complementary sequences.

<sup>28</sup> 26. The DNA of claim <sup>24</sup> 22, wherein at least one nucleotide is replaced by different nucleotide, on the basis of genetic degeneracy, without changing the amino acid sequence encoded thereby.

<sup>29</sup> 27. The DNA of claim <sup>24</sup> 22, which is inserted into an autonomously replicable vector.

<sup>30</sup> 28. The DNA of claims <sup>24</sup> 22, which is introduced into a host selected from the group consisting of animal, plant, and microbial hosts.

<sup>31</sup> 29. A process of producing a peptide comprising allowing a DNA that codes for the peptide of claim 1 to express and collecting the expressed peptide.

<sup>32</sup> 30. The process of claim <sup>31</sup> 29, wherein the peptide is collected by one or more techniques selected from the group consisting of salting out, dialysis, filtration, concentration, separatory sedimentation, ion-exchange chromatography, gel filtration chromatography, absorption chromatography, isoelectric-focusing chromatography, hydrophobic chromatography, reversed phase chromatography, affinity chromatography, gel electrophoresis, and isoelectric-focusing electrophoresis.

<sup>33</sup> 31. A process of preparing a peptide according to claim 1, comprising:

(a) preparing cells that produce antibodies against IL-18 consisting of the amino acid sequence of SEQ ID NO:21 or 22;

(b) cloning cDNAs for light and heavy chain variable regions from the antibody-producing cells prepared in step (a);

(c) constructing DNAs coding for single chain variable region fragments (scFvs) comprising a part or the whole of each cDNA cloned in step (b);

(d) expressing scFvs from the DNAs constructed in step (c);

(e) testing the scFvs expressed in step (d) on IL-18-neutralizing activity per antigen-binding site in comparison with an immunoglobulin molecule comprising the amino acid sequences of SEQ ID NOs:1 and 2 as the light and heavy chain variable regions respectively;

(f) selecting a DNA expressing an scFv that exhibits IL-18-neutralizing activity per antigen-binding site at a level not lower than that of the immunoglobulin molecule in step (e);

(g) constructing a DNA coding for scFv or humanized antibody as a peptide according to claim 1 with the DNA selected in step (f) and optionally with a foreign DNA, and inserting said constructed DNA into a vector;

(h) expressing the scFv or humanized antibody from the vector prepared in step (g); and

(i) collecting the scFv or humanized antibody from the resulting mixture of step (h);

34-32. A process of preparing a pharmaceutical composition for a disease selected from the group consisting of asthma, graft-versus host disease, rheumatoid arthritis, and sepsis, said process comprising:

(a) mixing a physiologically acceptable carrier with the peptide of claim 1 as an effective ingredient; and

(b) formulating the resulting mixture into a formula suitable for medicating a living body.

<sup>35</sup> 33. An agent for susceptible diseases, which comprises the peptide of claim 1 as an effective ingredient.

<sup>36</sup> 34. An agent of claim <sup>35</sup> 33, which further comprises a pharmaceutically acceptable carrier.

<sup>37</sup> 35. A method to treat a living body for preventing, alleviating, or remedying a disease selected from the group consisting of asthma, graft-versus-host disease, rheumatoid arthritis, and sepsis, said method comprising administering an effective amount of the agent of claim <sup>36</sup> 34 to the living body.

<sup>38</sup> 36. A method to treat a living body in need of autoimmunity, immunosuppressive, or anti-inflammatory treatment; said method comprising administering an effective amount of the agent of claim <sup>36</sup> 34 to the living body.

<sup>39</sup> 37. The agent of claim <sup>35</sup> 33, which contains as a stabilizer one or more members selected from the group consisting of albumin, saccharides, and buffers.

<sup>40</sup> 38. The agent of claim <sup>35</sup> 33, which is as an agent for auto-immune diseases.

<sup>41</sup> 39. The agent of claim <sup>35</sup> 33, which is an immunosuppressant.

<sup>42</sup> 40. The agent of claim <sup>35</sup> 33, which is an anti-inflammation agent.

<sup>43</sup> 41. An interleukin-18 neutralizer, which comprises the peptide of claim 1 as an effective ingredient.

~~44~~ 42. A method of neutralizing interleukin-18, which comprises allowing the peptide of claim 1 to act on interleukin-18.

~~45~~ 43. An interleukin-18 inhibitor, which comprises the peptide of claim 1 as an effective ingredient.

~~46~~ 44. A method of inhibiting interleukin-18, which comprises allowing the peptide of claim 1 to act on interleukin-18.

Add B<sup>1</sup>